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On the Instability of Brilliant Cresyl Blue.

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The employment of this dye (dimethyl diamino toluphenazine chloride) in biochemical work warrants the publication of this note on some of its properties.

The purified *dry* dye when extracted with *dry* ether, benzene or xylene gives no coloration to the solvent. Chloroform has the disadvantage that the dye salt is appreciably soluble in it.

Addition of water to the dye-solvent mixture results within 10 minutes in coloration of the non-aqueous phase. This coloration increases progressively with time, with increased temperature and with extremes of pH. It is not a result of interaction of dye with the non-aqueous solvent, for independent oxidation-reduction titrations in aqueous solutions show the presence and accumulation of secondary systems as contact of dye with water is prolonged. The conclusion must be, therefore, that *brilliant cresyl blue remains 'pure' only so long as it is kept out of contact with water.* This instability seems to be of the same kind and degree as that found in methylene blue.

The nature of the secondary products is unknown. It is suspected that they are demethylated and deaminated derivatives of the parent dye. At least 2 are present. One has the properties of an oxazone. It is a very weak base with dissociation constant less than 10^{-13} and its solutions from pH 1 up to normal KOH are colored red with a golden fluorescence. The second product has the properties of a stronger base, with a dissociation constant near 10^{-6}.* Its salt is colored blue and the base orange.

Brilliant cresyl blue itself is a stronger base with a dissociation constant of 10^{-3}.

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* This corresponds to the value assigned by Irwin, *J. Gen. Physiol.*, 1925, **9**, 561, to the dissociation constant of brilliant cresyl blue.